IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Miri Seiberg et al.

Serial No. 09/206,249

Filed: December 7, 1998

Art Unit: 1655

Examiner: Michael V. Meller

Attorney Docket No.: JBP-438

Title: METHODS FOR REGULATING PHAGOCYTOSIS AND ICAM-1 EXPRESSION

DECLARATION OF YAPING HU, PH.D.

I, Yaping Hu, am a Principal Scientist in the Skin Research Center at Johnson & Johnson Consumer Companies, Inc. My education includes a Ph.D. in biology from Rutgers University, NJ, and a B. S. in biology from Jimei University, Xiamen, China. My curriculum vita is attached hereto as Exhibit 1.

I hereby declare:

1. This Declaration is respectfully submitted to describe the trypsin inhibitory activity of soy compositions made in accordance with the above-captioned patent application and in accordance with the publication of "Matsuura" (JP 408143442).

2. In accordance with the data set forth in paragraph 5 below, the soybean compositions made in accordance with Matsuura exhibited minimal to no trypsin inhibitory activity. The soybean composition made in accordance with the above captioned patent application exhibited trypsin inhibitory activity of about 60% inhibition. This conclusion is based upon the experiment set forth in sections 3-5 below.

3. In order to create soy compositions in accordance with the Matsuura publication, I purchased premium dried soybeans (Hua-Mei Brand) from the Great Wall oriental food supermarket in South Brunswick, NJ, and made the following soybean preparations:
U.S. Patent Application Serial No. 09/206,249:

I soaked 20 gr whole soybeans in 200 ml water overnight at 20C and then ground the soaked beans in a warring blender for two minutes. This resulted in a preparation of 10% concentration of soybean material. I diluted the preparation to 1% for use in the trypsin inhibition assay due to the high trypsin inhibitory activity of this preparation.

I then replicated the following examples set forth in the Matsuura publication, as follows:

Examples A, B

I dry-heated whole soy beans at 75°C for two hours, then pressed and dehulled the beans. I ground 20g of of dehulled beans in a warring blender for 4 min with 200ml cooled water (5°C), and then heated the slurry to 100°C for 30 seconds. Preparations of Examples A and B are of 10% and 1%.

Examples C, D and E

I prepared samples as described in Example A and then sterilized them at 131°C for three minutes. The preparation of Example C had a concentration of 10%. The preparation of Example D had a concentration of 1%. For the preparation of Example E, the protein levels were adjusted to 3.5%.

Example F

I soaked 20 gr whole soybeans in 200 ml water for 5 minutes at 5°C and reserved the soaking liquid for evaluation.

Example G

I soaked 20 gr whole soybeans in 200 ml water for 5 minutes at 100°C and reserved the soaking liquid for evaluation.

Example H

I soaked 20 gr whole soybeans were soaked in 200 ml water for 20 hours at 5°C and reserved the soaking liquid for evaluation.

Example I

I soaked 20 gr whole soybeans in 200 ml water for 20 hours at 100°C and reserved the soaking liquid for evaluation.

Example J

I soaked 20 gr whole soybeans in 200 ml water for 20 hours at 20°C and reserved the soaking liquid for evaluation.

Example K

I soaked 20g whole soybeans in 200ml water at 5°C overnight, and then ground them in a warring blender for two minutes at 20°C. I then heated the slurry for five minutes at 75°C, and then reheated it to 100°C for five minutes.

I measured the inhibition of trypsin-induced cleavage of a fluorescent casein peptide using the EnzChek™ protease assay kit, following the manufacturer's instructions (EnzChek™ Protease Assay Kits Product Information, Molecular Probes, Eugene OR). Soy preparations were diluted in water as needed, and were incubated with 100 units of trypsin (Sigma, St. Louis, MO) dissolved in digestion buffer provided in the assay kit. A pure serine protease inhibitor (soybean trypsin inhibitor, from Sigma, St. Louis, MO) was used as a positive control at 0.05%, and 0.005% w/v. Then, 1.0 mg/ml stock solution of BODIPY FL casein was prepared by adding 0.2 mL of deionized water to the vials supplied with this substrate (provided in kit), then made to a final working concentration of 10 g/ml in digestion buffer. Following incubation of the trypsin, with or without the test material, with the BODIPY fluorescent casein substrate at room temperature for one and half hour, fluorescence was measured (excitation 485 nm /emission 530 (520) nm) on a SpectraMax® Gemini microtiter plate reader (Molecular Devices Corporation, Sunnyvale, CA) using Softmax® Pro 3.0 software (Molecular Devices Corporation).

4. The trypsin inhibition assay described above has some limitations. When the same preparation is tested with the same assay, independently, at different times, the results are not identical but appear within a range, which is a situation more commonly observed for samples with low inhibitory activity. For example, when the same pure STI preparation from Sigma was tested three times independently, using the same batch, same concentration, and same assay, the results that were obtained varied, as shown in Table 1 below. Because of this assay variability, results of this assay that are at or below 18-20% are considered as below the margins of detection of the reliable threshold to describe real biological activity.

Table 1

Sample	Trypsin inhibitory activity	Date of Assay
STI .005%	33.6	6/2/2010
STI .005%	15.7	6/23/2010
STI .005%	17.7	5/20/2010

The left-hand and middle columns of Table 2 below, which may be found in the Specification of the originally-filed patent application Serial No. 09/206,249 at p. 32, l. 15-20, further document the phagocytosis inhibitory data of STI compositions set forth in the application as filed. The percent of inhibition may be calculated from the "% Ingestion" column by subtracting the % Ingestion from 100%. I have made these calculations and added them to Table 2 in the right column. As set forth in Table 2, when the biological activity (inhibition of phagocytosis) was measured at the time of patent application, the error bars of the different measurements were as high as +/- 15%. These data further suggest that activities at or below 15% do not represent real biological activity but a technical threshold.

Table 2

Treatment	% Ingestion	% inhibition
Untreated	100 +/- 12	0+/- 12
STI, 0.01%	76 +/- 15	24+/- 15
STI, 0.1%	55 +/- 14	45+/- 14
STI, 1%	41.6 +/- 11	58.4+/- 11

5. Table 3 below shows that soy preparation prepared according to patent application Serial No. 09/206,249, and pure STI from Sigma, exhibit trypsin inhibition activity. In contrast, soy preparations made according to Matsuura have minimal to no trypsin inhibition activity. The preparations tested in Table 3 were of different concentrations, therefore the level of trypsin inhibitory activity per 1% soy preparation was calculated from the raw data. The soy preparation of patent application Serial No. 09/206,249 (Attorney Docket No. JBP438), at 1% concentration, had inhibitory activity of 57.9%. This is much higher than

the 18-20% cutoff, which is considered as "noise" and is not believed to be related to real biological activity. All the Matsuura preparations (A-K) showed no trypsin inhibitory activity above this cutoff, documenting that they lack trypsin inhibitory activity. The graphed results of Table 2 are set forth in Exhibit 2 attached hereto.

Table 3

Samples	% Inhibition of Trypsin of sample	% trypsin inhibition of 1% soy preparation, calculated
STI at 0.05 %	84	-
STI at 0.005%	15.6	-
09/206,249 Soy, 1%	57.9	57.9
A	0.0	0.0
B	1.3	1.3
C	3.3	0.33
D	-3.8	-1.25
E	6.3	6.3
F	7.4	0.74
G	-33.6	-3.3
H	-26.4	-2.6
I	6.7	0.67
J	-15.9	-1.5
K	15.2	15.2

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Yaping Hu Yaping Hu

Date 6/30/2010

Exhibit 1

CURRICULUM VITAE

Ya-Ping Hu

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Skillman, NJ 08558
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Education:

Ph.D., Biological Science, Rutgers University. 1992.
M.S., Biological Science, Chinese Academia of Sciences. 1982.
B.S., Biology, Jimei University, Xia Men, China. 1976.

Professional Experience:

06/2007- recent, Principal Scientist, Johnson and Johnson, Skin Research Center Skillman, NJ 08558.

- Development of in vitro skin explants model for skin research.
- Skin pigmentation.
- Skin aging.

04/1999-05/2007, Research and Teaching Specialist II, Center for Advanced Biotechnology and Medicine, UMDNJ, Piscataway, NJ.

- Create mutant mouse model for study of gene functions.
- Embryonic stem cell (ESC) culture, maintaining various mutant ESC strains and stocks;
- Training the grad-students and post-docts in molecular cloning, gene targeting strategy and ESC culture techniques.

05/1997-04/1999, Research Associate, Dept. of Surgery, UMDNJ, Piscataway, NJ.

- Using *in vitro* model to study the cell-matrix and cell-material interaction.
- Application of molecular biology techniques to characterize the platelet ahpha-actinin. Generated GFP and His-tagged alpha-actinin fusion proteins, point and truncation mutants, expression of fusion protein in bacteria and mammalian cells.

05/1995-05/1997, Postdoctoral Fellow, Center for Human and Molecular Genetics, UMDNJ, Newark, NJ.

- Positional cloning, mapping and linkage analysis of human non syndrome deafness. Research efforts involve handling of blood samples collected from several families and genotyping with chromosome markers.

08/1992-05/1995, Postdoctoral Researcher, Molecular Evolution Program, Louisiana State University, Baton Rouge, LA. (8/92-5/95; Dr. David Foltz)

- Developed single copy DNA markers for species and population identification.
- Investigation of population genetics in early life stages and geographic populations of marine organisms

01/1987-06/1992, Graduate Student, Center for Theoretical and Applied Genetics, Rutgers University, New Brunswick, NJ),.

- Application of molecular biology techniques to species identification and genetics analysis.
- Genetics study including *in vitro* fertilization, embryo culture, polypolyploid induction and karyotyping.

Publications:

Retinol induces dermal elastin synthesis and elastin fiber formation. D. Rossetti, M. G. Kielmanowicz, S. Vigodman, Y. Hu, N. Chen, A. Nkengne, T. Oddos, D. Fischer, M. Seiberg and C. B. Lin, Int. J.of Cosmetic Science, in press (available online), 2010.

Characterization and Immunohistochemical Evaluation of Solar Lentigines: the Involvement of KGF/KGFR and Other Factors in Lesion Development. Connie B. Lin, Yaping Hu, Dianne Rossetti, Nannan Chen, David Cassarino, Andrzej Slominski, and Miri Seiberg International Dermatological Science, accepted for publication, 2010.

A luminal epithelial stem cell that is a cell of origin for prostate cancer. Xi Wang, Marianna Kruithof-de Julio, Kyriakos D. Economides, David Walker, Hailong Yu, M. Vivienne Halili, Ya-Ping Hu, Sandy M. Price, Cory Abate-Shen & Michael M. Shen. (Nature 461: 495-500, 2009).

Context-dependent neuronal differentiation and germ layer induction of Smad4-/- and Cripto-/- embryonic stem cells. Sonntag KC, Simantov R, Bjorklund L, Cooper O, Pruszak J, Kowalke F, Gilmartin J, Ding J, Hu YP, Shen MM, Isacson O. Mol Cell Neurosci. Mar;28(3):417-29, 2005

The cytoskeletal/non-muscle isoform of alpha-actinin is phosphorylated on its actin-binding domain by the focal adhesion kinase. Izaguirre G, Aguirre L, Hu YP, Lee HY, Schlaepfer DD, Aneskievich BJ, Haimovich BJ Biol Chem. 276(31):28676-85, 2001.

A point mutation in the CytB gene of mtDNA associated with complex III deficiency in ischemic cardiomyopathy. Marin-Garcia, J., Y.P. Hu, R. Ananthakrishnan, M.E. Pierpont, G.L. pierpont and M. J. Goldenthal. Biochemistry and Molecular Biology

International, 40(3): 487-495, 1996.

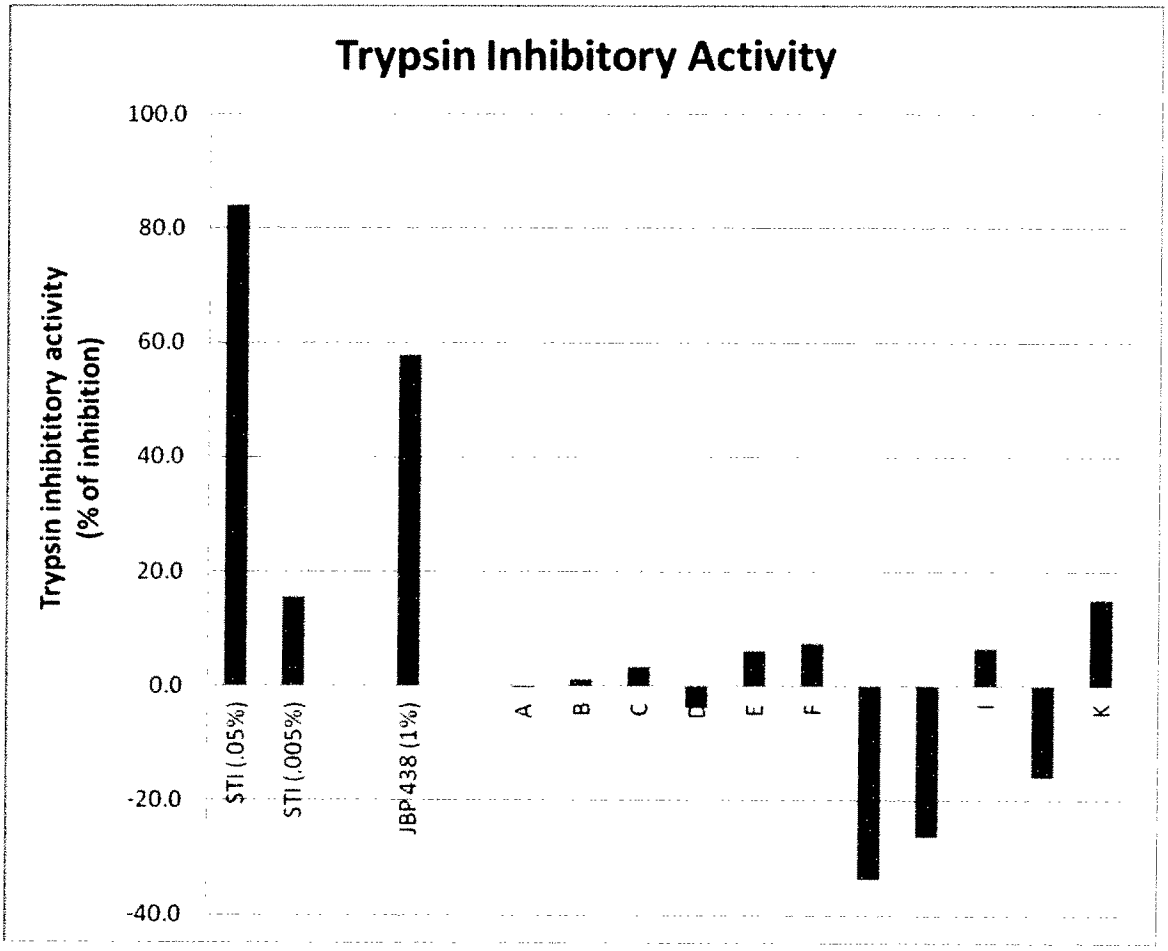
Genetics of scnDNA polymorphism in juvenile oysters, Crassostrea virginica: Inheritance and analysis of PCR-RFLPs in controlled crosses. Hu, Y.P. and D.W. Foltz. Molecular Marine Biology and Biotechnology, 5(2):123-129, 1996.

Genetics of scnDNA polymorphism in juvenile oysters, Crassostrea virginica: heterozygosity deficiency and linkage disequilibrium in natural population samples. Foltz, D.W. and Y.P. Hu. Molecular Marine Biology and Biotechnology, 5(2): 130-137, 1996.

Overdominance in early life stages of an inbred American oyster. Hu, Y.P., R.C. Vrijenhoek and R.A. Lutz. J. Heredity, 84: 254-258, 1993.

Electrophoresis and genetic analysis of larval bivalve mollusks. Hu, Y.P., R.A. Lutz and R.C. Vrijenhoek. Mar. Biol. 113: 227-230, 1992.

Exhibit 2


Trypsin Inhibitory Activity
Trypsin inhibititory activity (% of inhibition)
100.0
80.0
60.0
40.0
20.0
0.0
-20.0
-40.0
STI (.05%)
STI (.005%)
JBP438 (1%)
A
B
C
D
E
F
I
K